HYPERSENSITIVITY TO THE CHOLINESTERASE INHIBITOR DI-*iso*-PROPOXY-PHOSPHORYL FLUORIDE (DFP)§

Cross-sensitization to Related Compounds

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Since Schrader (1) began to synthesize alkyl-phosphates, a great many organo-phosphorus compounds have been produced. As these are cholinesterase inhibitors, some of them have been used as insecticides and the most toxic ones are regarded as potential war gases. A number of them have been put to use in medicine, *e.g.*, di-*iso*-propoxy-phosphoryl fluoride (DFP) which was synthesized by Saunders (2). This compound has been used in the treatment of glaucoma, of post-operative paralytic ileus, and of myasthenia gravis.

Although glaucoma patients often use eye-drops containing DPF for daily conjunctival instillation for months and even years, only few reports on allergic reactions to such drops have so far been published. Stone (3) observed two patients with sensitivity to DFP in which "cutaneous tests" were positive to the drug in peanut oil and negative to the vehicle. He also refers to a case reported by Guerry with the same response to "cutaneous tests." In other reports on presumed hypersensitivity to DFP, no epicutaneous testing seems to have been performed. In addition to systemic side-effects of other organo-phosphorus cholinesterase inhibitors, skin reactions have also been reported (4). In these reports, which only deal with the insecticide group, hypersensitivity has in no case been demonstrated on epicutaneous testing.

We have observed a patient with eczema of the eye-lids associated with instillation of eye-drops containing DFP. The hypersensitivity to DFP was demonstrated by positive reaction on epicutaneous testing. In order to establish a possible group specificity of this hypersensitivity, the patient was tested with some chemically related compounds.

§ Presented at the Meeting of the Southwestern Swedish Dermatologists, Gothenburg, May 27, 1961.

Received for publication July 18, 1961.

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CASE REPORT

Female, aged 63. Family history—Mother urticaria, brother bronchial asthma. Previous illnesses—No history of eczema or other allergic diseases. Glaucoma for four years, topically treated with a solution of pilocarpine and physostigmine, and with Floropryl (0.1 per cent di-*iso*-propoxy-phosphoryl fluoride in peanut oil, Merck Sharp & Dohme) for the last year.

Present illness—At the first examination, the patient presented an eczema of the right eyelid. The eczema had developed slowly during the last five months. It receded slowly after discontinuing the use of the Floropryl drops, and on application of a local hydrocortisone remedy. Routine epicutaneous patch testing was performed without any positive reactions. The patient was also tested with the eye-drops used, *i.e.*, the pilocarpine-physostigmine, and Floropryl. Only Floropryl gave an eczematous reaction. Subsequent testing with the two components of Floropryl* was performed. An 1/10,000 dilution of DFP in olive oil gave an eczematous reaction, while peanut oil and olive oil gave no such reactions.

INVESTIGATION OF SENSITIVITY

In order to analyze the importance of the fluorine atom and of the alkoxy groups for the specificity of the hypersensitivity to DFP, the patient was tested with compounds in which these radicals had been substituted. As the desired compounds were difficult to obtain, the cross-sensitization pattern could not be completely analyzed. We succeeded in securing 17 compounds for the epicutaneous testing.§§ All these compounds were applied in a concentration of 1/10,000 in olive oil. When negative, also a concentration of 1/1,000 was tried. Because of the high toxicity of methyl-*iso*-propoxy-phosphoryl fluoride (Sarin), the highest test concentration of this substance was 1/5,000. Two drops (40 mg) were applied on the test patch.

* The supply of pure DFP and of peanut oil, by Merck Sharp & Dohme, New York, U.S.A., is gratefully acknowledged.

§§ The supply of compounds marked $^{++}$ in table 1, by The Research Institute of National Defence (Försvarets Forskningsanstalt), Stockholm, Sweden, and of compounds marked $^{+++}$, by dr G. Schrader, Farbenfabriken Bayer, Germany, is gratefully acknowledged.

Not more than three compounds were tested simultaneously. No cholinesterase inhibiting effects were observed during the testings.

The test reactions are given in table 1.

In addition to DFP, diethoxy-phosphoryl fluoride, *iso*-propoxy-*iso*-propyl-phosphoryl fluoride and ethoxy-(dimethylamino)-phosphoryl fluoride gave positive reactions. Thirteen compounds showed negative reactions.

Testing with several compounds which gave negative reactions but were chemically related to the four allergenic substances made it possible to determine the main structural requirements for the cross-reactivity.

The organic phosphorus compounds are easily hydrolyzed in the presence of water. It might be assumed that the allergic reaction to DFP was caused by some of the hydrolyzation products obtained through contact with the skin. However, a hydrolysate of DFP (table 1, b) containing di-*iso*-propoxy-phosphate and hydrofluoric acid gave a negative reaction.

Testing with a compound in which fluorine in DFP had been replaced by chlorine gave a negative reaction. Furthermore, the chloride, the amide, and the cyanide (table 1, d) corresponding to the allergenic diethoxy-phosphoryl fluoride (table 1, e) gave negative reactions. Thus the fluorine atom seems to be necessary for the allergenicity.

In diethoxy-phosphoryl fluoride (table 1, e) both alkoxy groups contain one carbon atom less than those of DFP, yet a positive reaction was obtained. Whether a compound with only one carbon atom in each chain would give an allergic reaction, could not be determined as the corresponding dimethoxy compound was not available. With four or more carbon atoms in each chain (table 1, f) a negative reaction was obtained. Thus the length of the alkoxy groups—and through this the length of the whole molecule—appears to determine the allergenic properties. This finding is in accordance with the allergenic properties of certain diphenols which show cross-sensitization (5).

When the alkyls are replaced by aromatic nuclei (table 1, g) the allergenicity is lost.

Iso-propoxy-*iso*-propyl-phosphoryl fluoride (table 1, h) gave a positive reaction. Thus it was possible to exclude at least one oxygen atom of the alkoxy group. A compound in which both oxygen atoms were excluded was not available for the present investigation. When the two oxygen atoms are replaced by nitrogen, as in di-*iso*-propylamino-phosphoryl fluoride (table 1, i), the group specificity is lost.

Methyl-*iso*-propoxy-phosphoryl fluoride (table 1, j) yielded a negative reaction. This negative reaction might be due either to the shortness of one of the alkoxy groups or to the difference in length between them.

Finally, ethoxy-(dimethylamino)-phosphoryl fluoride (table 1, k) gave a positive reaction. This compound, like *iso*-propoxy-*iso*-propyl-phosphoryl fluoride (table 1, h), has slightly asymmetric carbon chains, but the shortest chain is longer than the methyl radical of the non-allergenic methyl-*iso*-propoxy-phosphoryl fluoride (table 1, j).

It was not possible to clarify the importance of the double-bound oxygen atom, as no compound was available in which the oxygen had been replaced by another element, *e.g.*, sulfur.

CONCLUSIONS

The eyelid eczema of the patient had a clear connection with the use of eye-drops containing di-*iso*-propoxy-phosphoryl fluoride (DFP). The hypersensitivity to the pure substance was demonstrated by epicutaneous testing; there was no hypersensitivity to compounds produced by hydrolysis.

The patient showed hypersensitivity to three compounds chemically related to DFP. These three compounds are synthetic and not in common use. Therefore, the patient cannot have had contact with them before. For this reason a true cross-sensitization with DFP as the primary allergen is present.

In addition to the central phosphorus atom, the structural requirements for cross-sensitization between these organo-phosphorus compounds appear to be

1. a fluorine atom.
2. two symmetric or slighty asymmetric aliphatic chains (alkyl, alkoxy or alkylamino groups), each containing not more than three carbon atoms.
3. an oxygen atom double-bound to the phosphorus atom.

There does not seem to be any relationship between the requirements of molecular structure for the allergenicity in this case and for the toxicity (6) of the compounds.

TABLE 1

Compounds tested

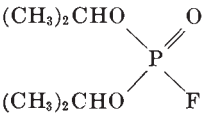
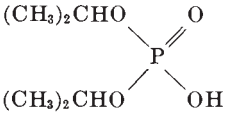
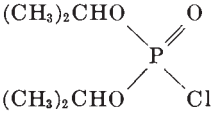
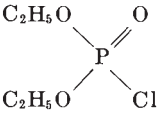
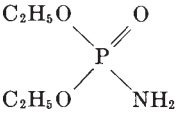
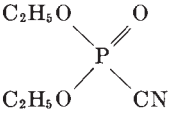
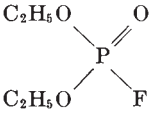
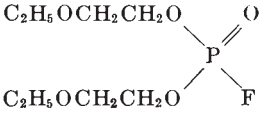
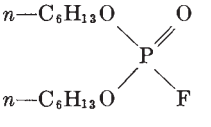
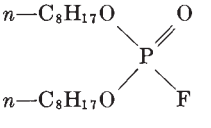
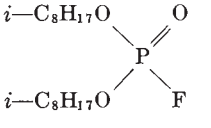
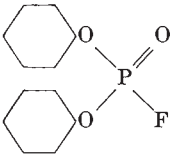
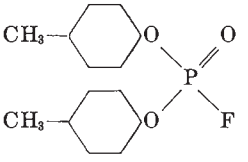
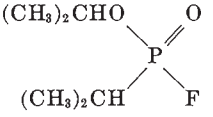
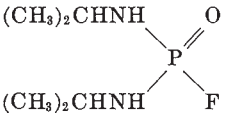
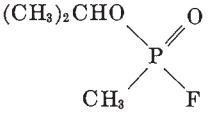
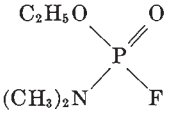
a	di-*iso*-propoxy-phosphoryl fluoride (DFP) (1/10,000)		*positive*
b	di-*iso*-propoxy phosphate (hydrolysate)$^{++}$ (1/1,000)		negative
c	di-*iso*-propoxy-phosphoryl chloride^{++} (1/1,000)		negative
d	diethoxy-phosphoryl chloride^{++} (1/1,000)		negative
	diethoxy-phosphoryl amide^{+++} (1/1,000)		negative
	diethoxy-phosphoryl cyanide^{+++} (1/1,000)		negative
e	diethoxy-phosphoryl fluoride^{++} (1/10,000)		*positive*
f	di-(ethoxy-ethoxy)-phosphoryl fluoride^{+++} (1/1,000)		negative
	di-*n*-hexoxy-phosphoryl fluoride^{+++} (1/1,000)		negative
	di-*n*-octoxy-phosphoryl fluoride^{+++} (1/1,000)		negative
	di-*iso*-octoxy-phosphoryl fluoride^{+++} (1/1,000)		negative

TABLE 1—*Continued*

g di-*cyclo*hexoxy-phosphoryl fluoride^{+++} (1/1,000)		negative
di-(4-methyl*cyclo*hexoxy)-phosphoryl fluoride^{+++} (1/1,000)		negative
h *iso*-propoxy-*iso*-propyl-phosphoryl fluoride^{++} (1/5,000)		*positive*
i di-*iso*-propylamino-phosphoryl fluoride^{+++} (1/1,000)		negative
j methyl-*iso*-propoxy-phosphoryl fluoride^{++} (1/5,000) (Sarin)		negative
k ethoxy-(dimethylamino)-phosphoryl fluoride^{+++} (1/5,000)		*positive*

SUMMARY

Epicutaneous allergy to the cholinesterase inhibitor di-*iso*-propoxy-phosphoryl fluoride (DFP) in a 63-year-old woman is described. An eyelid eczema had developed from the use of eye-drops containing DFP.

Cross-sensitization to diethoxy-phosphoryl fluoride, to *iso*-propoxy-*iso*-propyl-phosphoryl fluoride, and to ethoxy-(dimethylamino)-phosphoryl fluoride is demonstrated. The reactive compounds constitute a new group of substances demonstrating cross-sensitization. The structural requirements for the cross reactions are discussed.

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